



2.3.4.100 - Lignin Utilization

DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

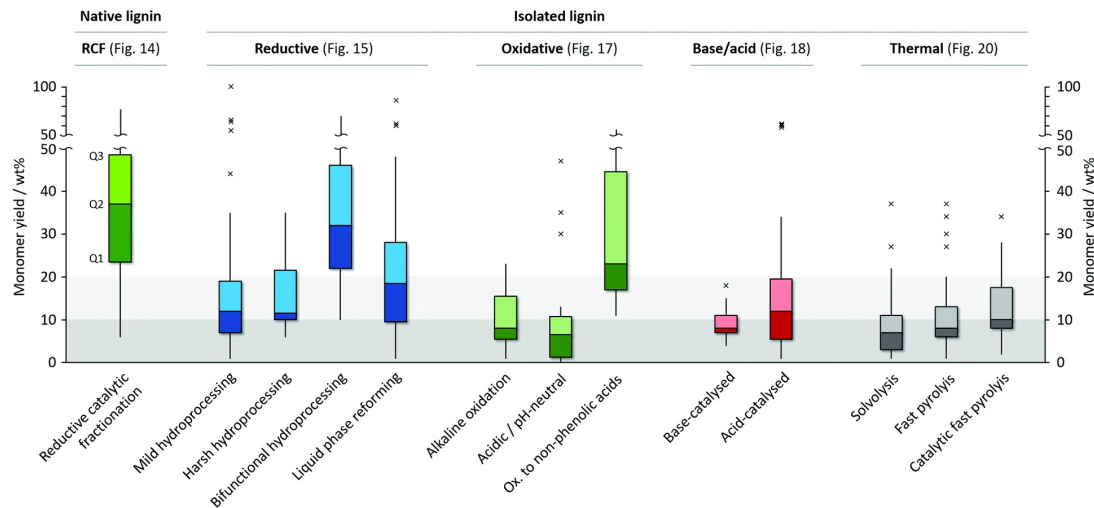
Technology Session Review Area: Biochemical Conversion & Lignin Valorization

PI: Gregg T. Beckham, National Renewable Energy Laboratory

Project overview

Goal: Develop industrially-relevant, feedstock-agnostic catalytic processes to generate aromatic monomers from lignin (and tools to characterize lignin)

- Project can enable **deconstruction-funneling paradigm**
- Deconstruction catalysis for **C–C bonds** in lignin – exceed monomer yield from C–O bond cleavage (~30-40%)
- Develop processes to provide high yields of aromatics to Biological Lignin Valorization (BLV) and Performance-Advantaged Bioproducts (PABP)
- Develop new lignin analytics and conduct model compound syntheses (down-scoped in FY23)



Schutyser *et al. Chem. Soc. Rev.* 2018

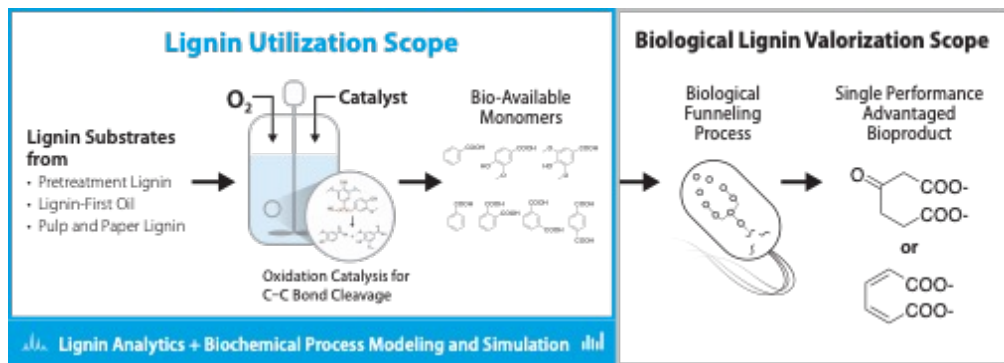
Technical approach

Lignin depolymerization

- Aerobic oxidation to make bio-available aromatics
- Leverage industrial oxidation processes
- Complementary to reductive methods (LigFirst/LigSAF)
- TEA and LCA with Biochem. Analysis project
- **Substrates:** hydrolysis lignin, technical lignins, RCF oil, HDO lignin
- Comprehensive **analytics** suite to characterize lignin in polymer/oligomer/monomer forms
- Work with SepCon for advanced separations
- Work with BLV and PABP to make products
- Collaborate with Shannon Stahl, UW Madison

Lignin analytics and model compound syntheses

- LC/MS-MS; work with Biochem. Process Modeling & Simulation for data analysis, posted on GitHub
- Bespoke model compound syntheses posted on www.protocols.io



Risks, management, and milestones

Risks:

- **Risk:** Stabilization chemistry is too expensive
- **Mitigation:** Examine routes that use acetic acid for stabilization, alternative C–C bond cleavage routes
- **Risk:** Supply chain issues delay reactor systems
- **Mitigation:** Collaborate with groups with existing flow-based oxidation setups (UW Madison)

Management, Communication, & DEI:

- Monthly project meetings, *ad hoc* meetings with LigFirst/BLV, open to all
- Use Dropbox for shared files
- Dedicated Project Manager
- Funding for early career staff to present and network
- Methods are open-source (protocols.io, GitHub, publications)
- Focused on physically and psychologically safe R&D environments

Milestones:

- **FY23:** >50% yield of bio-available aromatics
- **FY24:** >80% yield of bio-available aromatics
- **FY24 Go/No-Go:** Autoxidation on phenolic lignins (TEA/LCA)
- **FY25:** $\geq 80\%$ bio-available aromatics to BLV to produce a single compound with a \$2/GGE and 70% reduction in GHGs relative to fossil adipic acid

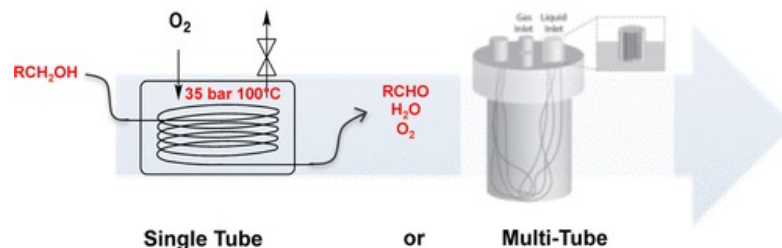
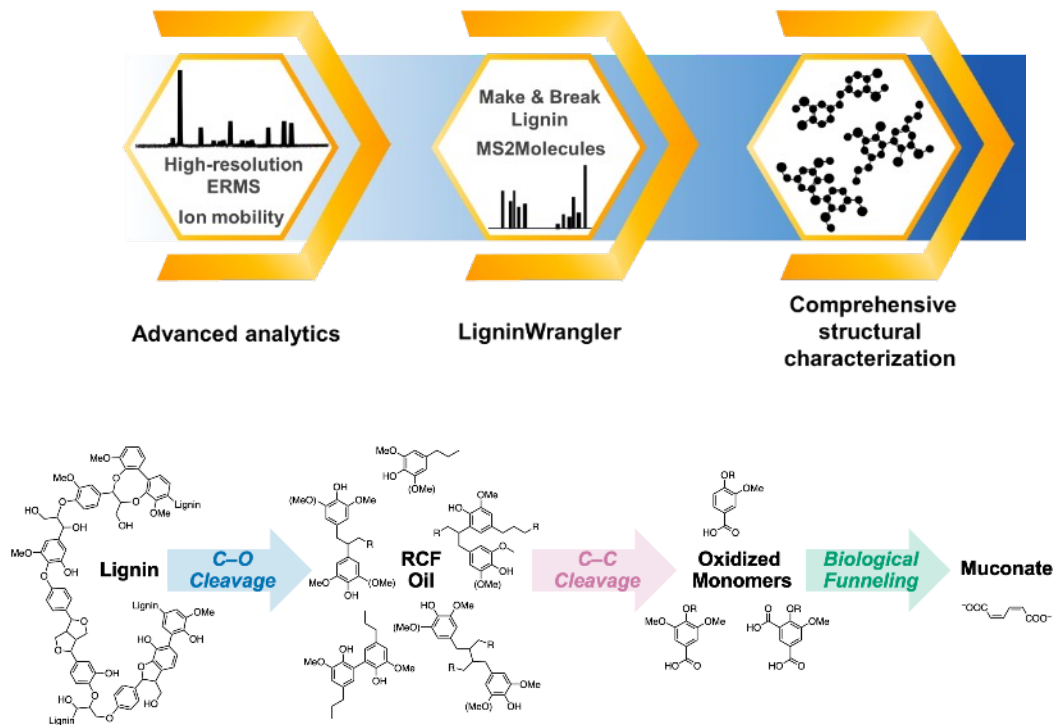


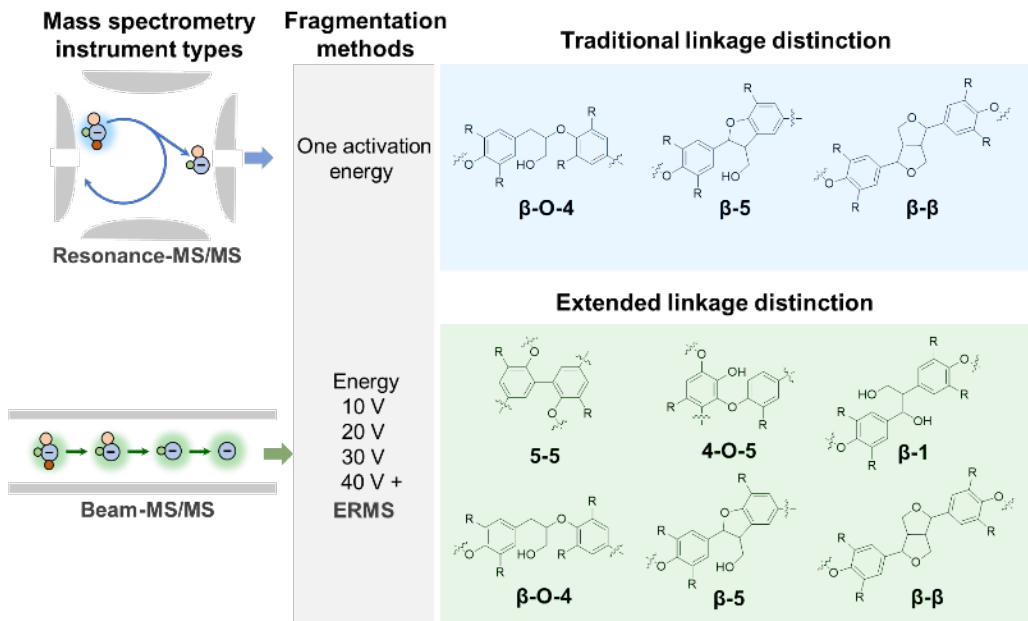
Image from Greene *et al.*, *OPRD* 2015

Outline of progress and outcomes

- Lignin analytics method development for tracking lignin in the biorefinery
- Automation of lignin MS data analysis with BPMS project
- Analytics and synthesis protocols
- Catalytic autoxidation for C–C bond cleavage in lignin oils
- Catalytic autoxidation of lignin HDO substrates

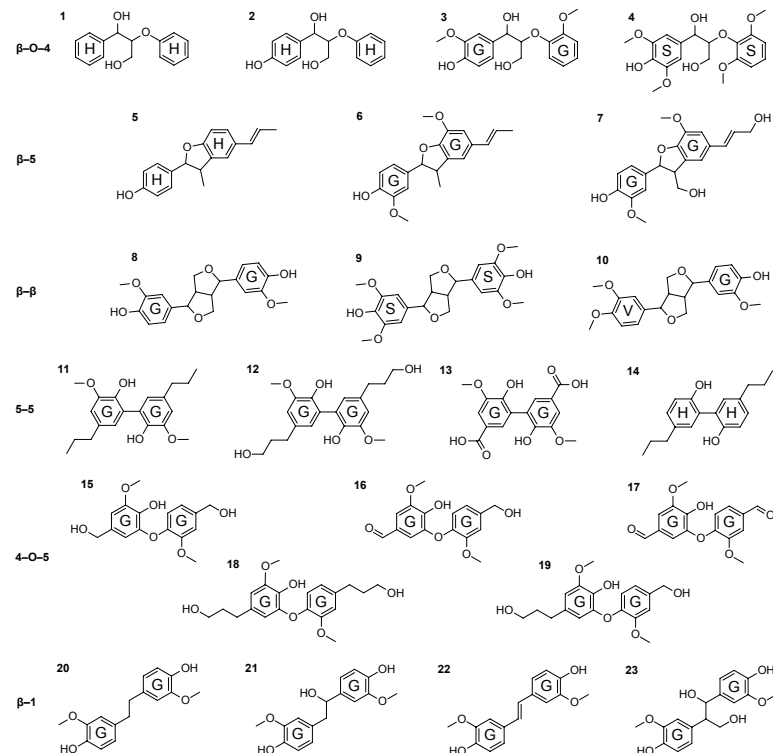


Advancing lignin analytics for linkage fingerprinting

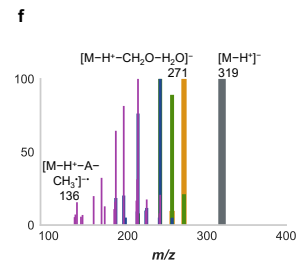
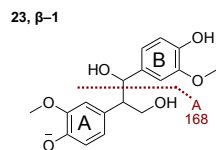
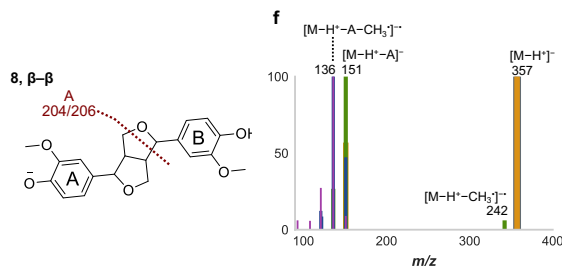
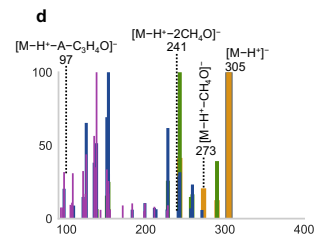
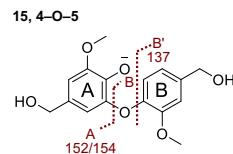
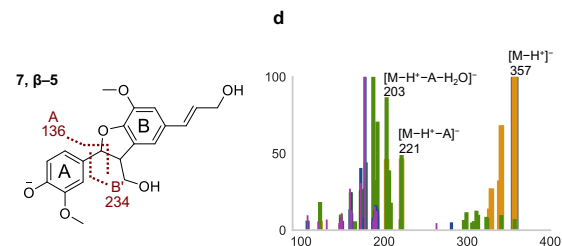
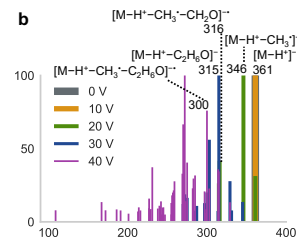
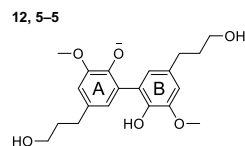
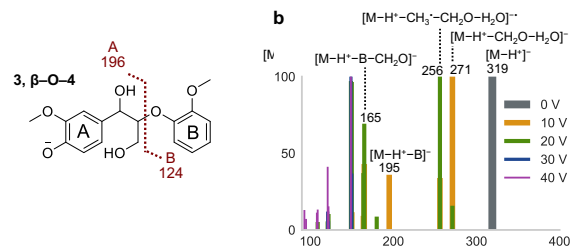


Beam-type energy-resolved MS enables fingerprinting of 6 major lignin linkages

- Can enable direct tracking of lignin compounds throughout a biorefinery process



Energy-resolved mass spectrometry provides a unique fingerprint for all lignin linkages



All 6 linkages provide distinct fingerprints – allows rapid identification of lignin chemistry from fragmentation pattern...

...and the fragmentation pattern analysis can feed into automated computational analysis...

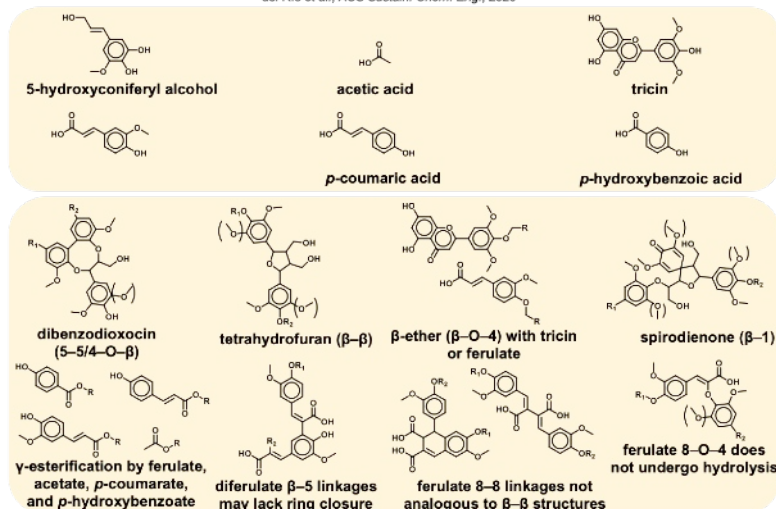
LigninWrangler allows automated LC/MS-MS analysis of lignin

Mayes, Black *et al.* in preparation

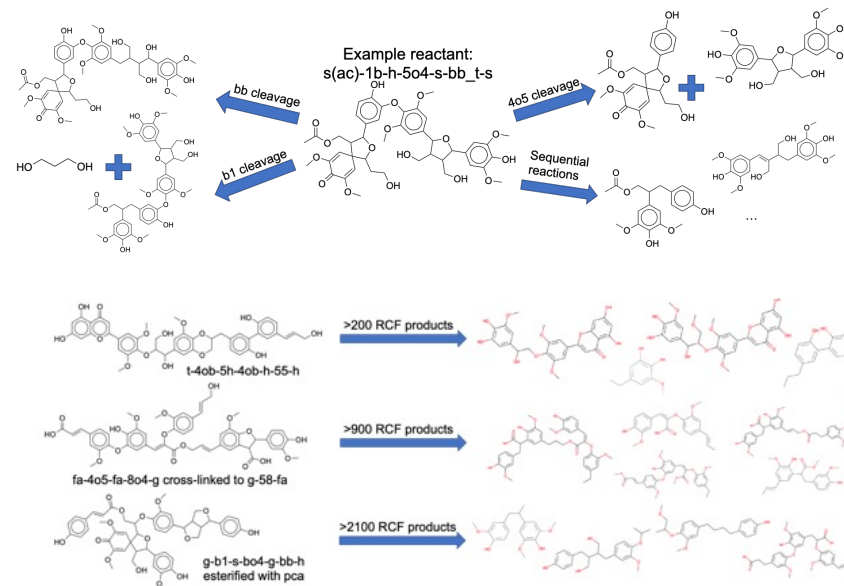
Module 1: make_lignin

New in LigninWrangler, to reflect current understanding of lignin composition

Ralph *et al.*, *Curr. Opin. Biotechnol.*, 2019
del Río *et al.*, *ACS Sustain. Chem. Eng.*, 2020



Module 2: break_lignin



Library constructed of ~21 million lignin compounds from reductive depolymerization to date...

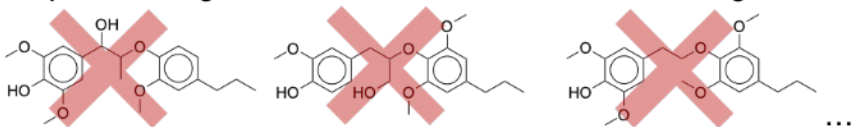
- Library contains most recent reported discoveries in lignin chemistry for maximum feedstock versatility
- Can be updated by any user and have user-fed libraries or user-fed constraints on library building

LigninWrangler Module 3: ms2molecules to narrow compound identification

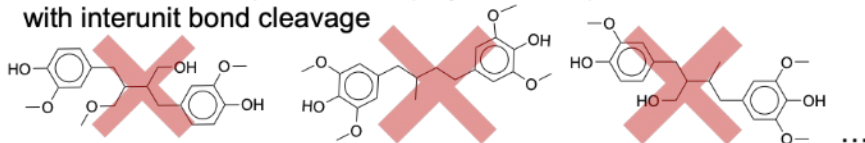
Example: Evaluation of high mass accuracy beam-ERMS MS/MS spectra from a RCF oil from a poplar feedstock

Evaluation of candidate $C_{21}H_{28}O_6$ in the default library:

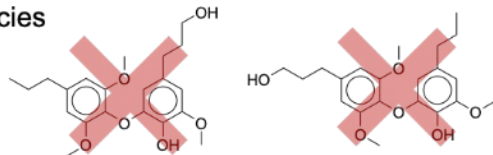
- All β -O-4 linkages ruled out due to lack of interunit cleavage at 10 V



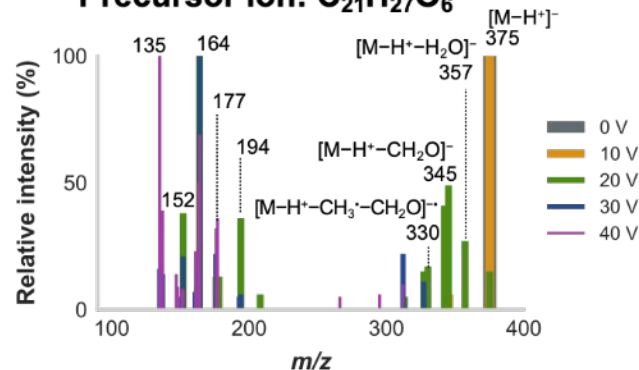
- There are no candidate 5-5 or β -1 linked species with this formula
- All β - β linked structures with this molecular formula are ring-opened, which would be expected to display far fewer product ions consistent with interunit bond cleavage



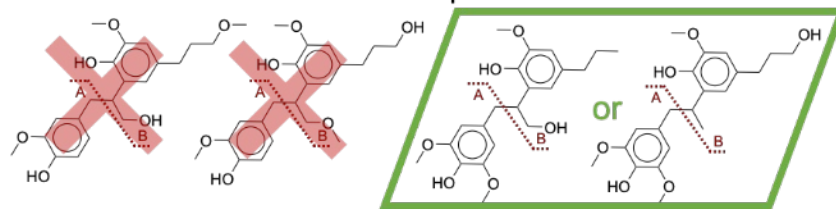
- The ~100 Da gap between lower and higher product ion m/z values is inconsistent with expected fragmentation of candidate 4-O-5 linked species



Precursor ion: $C_{21}H_{27}O_6^-$



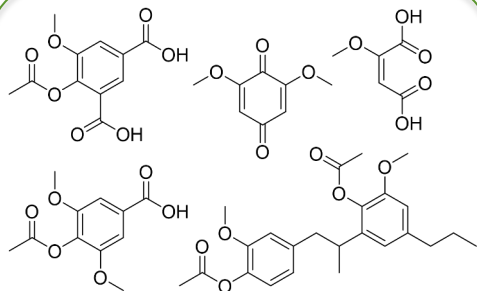
- All candidate β -5 structures are ring-opened, which, as a proposed intermediate in phenylcoumaran cleavage (Morreel *et al.*, *Anal. Chem.*, 2010), are expected to undergo linkage cleavage. The expected resulting fragment masses are consistent with two of the four options.



Allows for rapid, automated identification of compound chemistry in a high-throughput manner

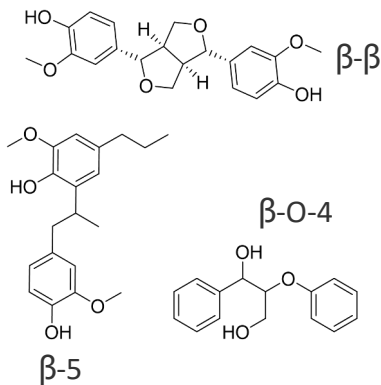


Protocols for synthesis and analytics



Exemplary compounds for analytical quantitation

Exemplary model compounds for synthesis

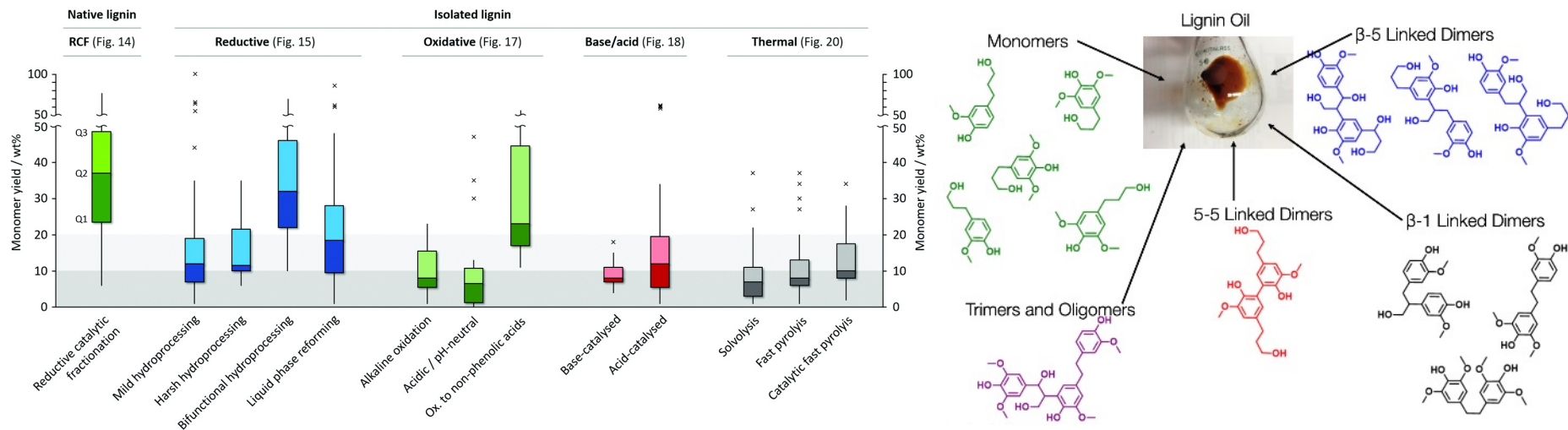


- Lignin Reductive Catalytic Fractionation (RCF) Acetylated Monomers Analysis by Gas Chromatography Flame Ionization Detection (GC-FID)
- Acetylated Products from Lignin Autooxidation by Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS)
- Lignin Reductive Catalytic Fractionation (RCF) Methylated Oxidation Products by Ultra High Performance Liquid Chromatography (UHPLC)
- Lignin Reductive Catalytic Fractionation (RCF) Monomers Analysis by Gas Chromatography Flame Ionization Detection (GC-FID)
- Synthetic Procedure of 2-(1-(4-hydroxy-3-methoxyphenyl)propan-2-yl)-6-methoxy-4-propylphenol
- Synthetic Procedure of Pinoresinol
- Synthetic Procedure of 2-Phenoxy-1-phenylethanol (PE)

protocols.io allows for an open-source collection of step-wise protocols with aim towards reproducibility

- Published multiple analytics and model compound syntheses online for the community to use

Carbon–carbon bond cleavage in lignin is a major technical hurdle

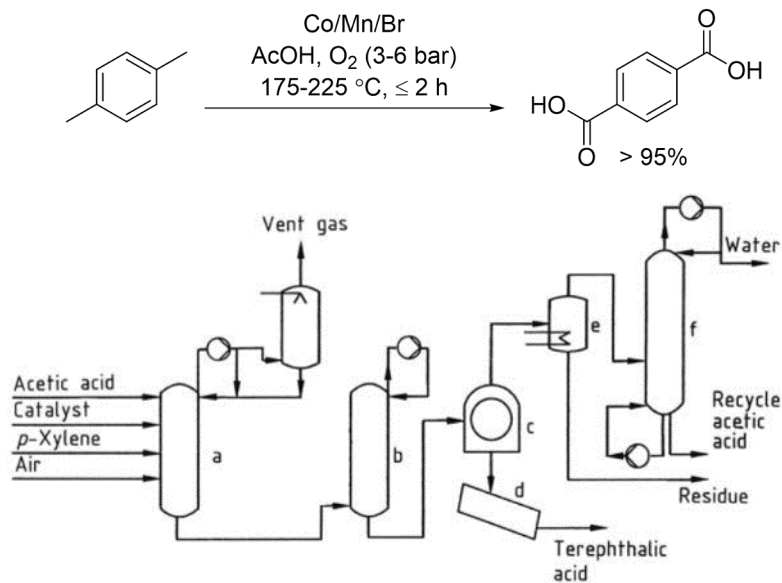


Lignin depolymerization yields from most optimal methods usually only ~30-40%

- Refractory carbon–carbon bonds in lignin inherently limit aromatic monomer yields

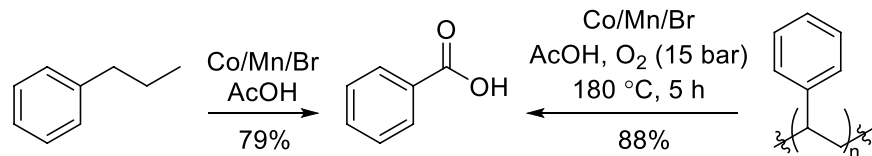
Adapting industrial autoxidation catalysis for C–C bond cleavage in lignin

Amoco process:

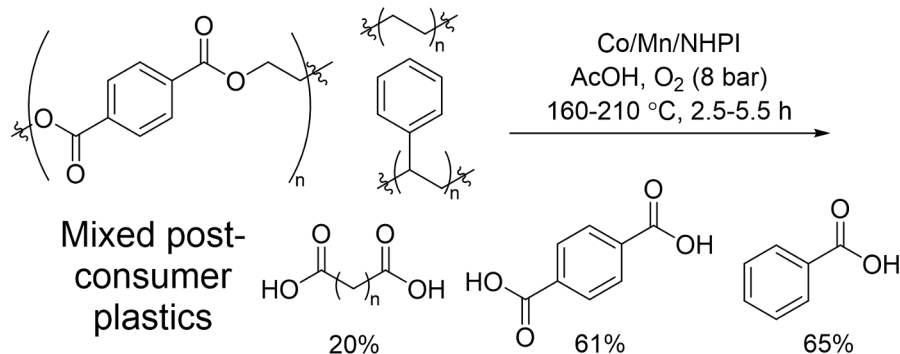


Sheehan, *Ullmann's Encyclopedia*, 2000

Previous studies of C–C bond cleavage:



Partenheimer, *Catal. Today*, 2000; Partenheimer, *Catal. Today* 2003

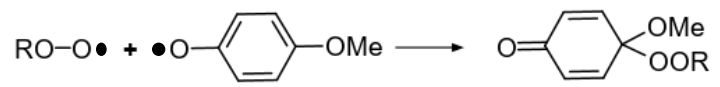
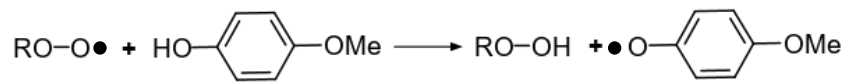
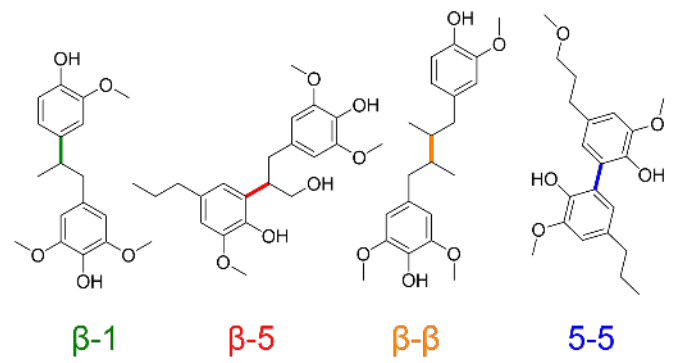


Sullivan et al. *Science*, 2022

Co/Mn/Br oxidation with O₂ in acetic acid is effective for C–C bond cleavage in hydrocarbon substrates

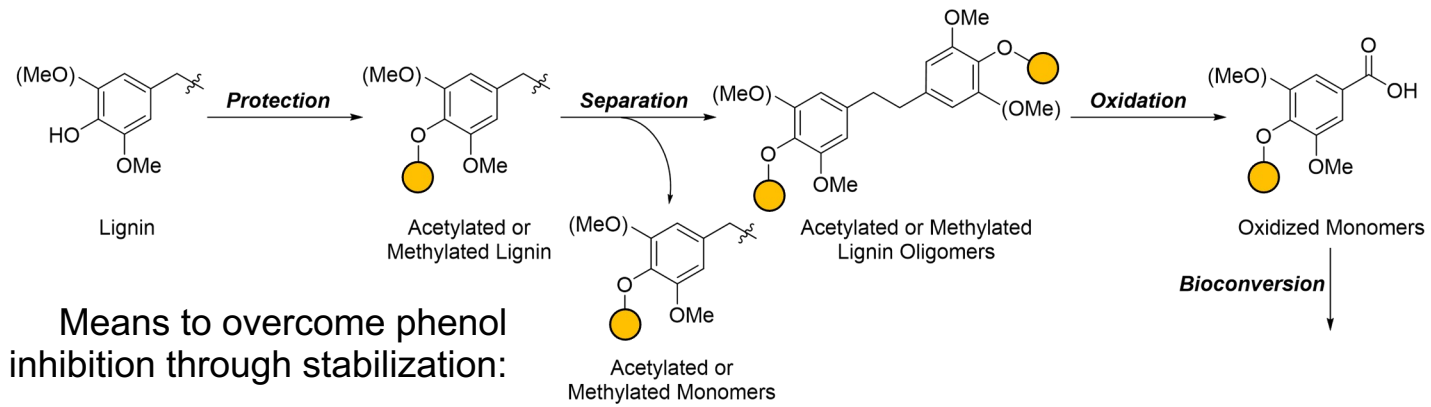
- Substantial industrial precedent for this chemistry in terephthalic acid manufacturing

Phenols are inhibitory to autoxidation



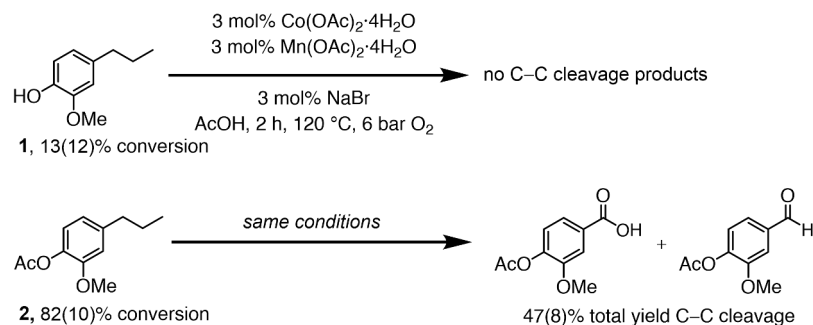
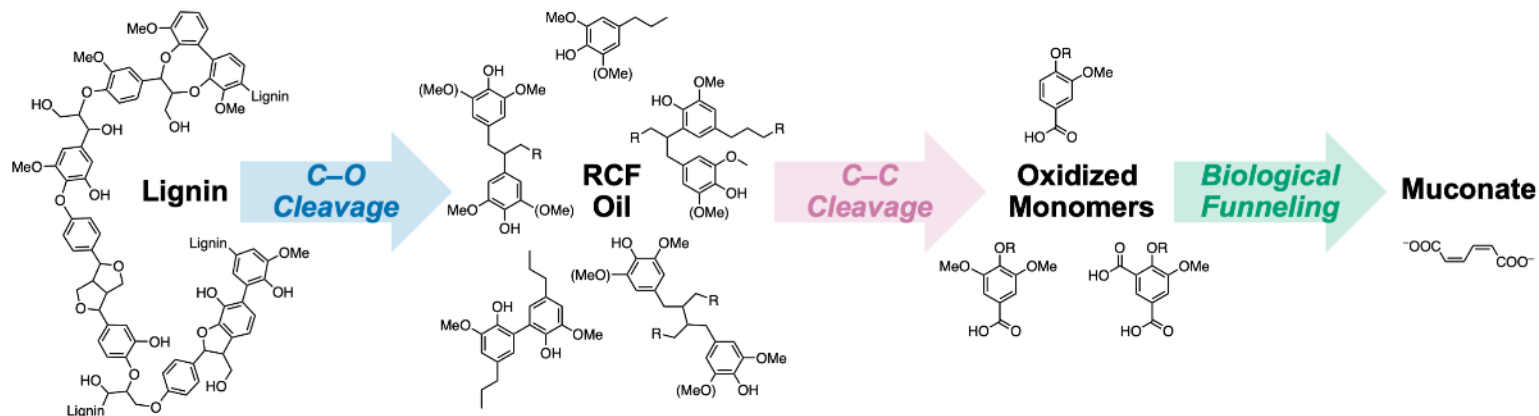
Lucarini & Pedulli, *Chem. Soc. Rev.* 2010

Exemplary C–C bonds in lignin that catalytic approaches must cleave

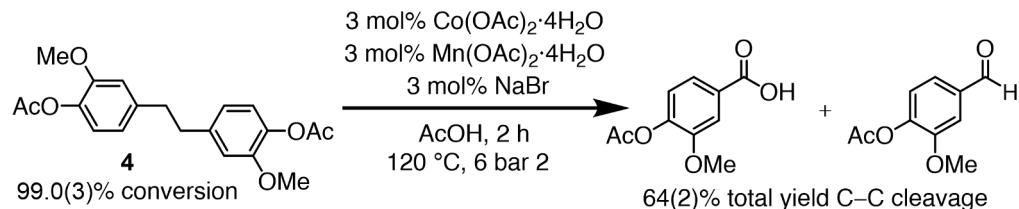


Means to overcome phenol inhibition through stabilization:

Acetyl stabilization of phenols leads to effective C–C bond cleavage

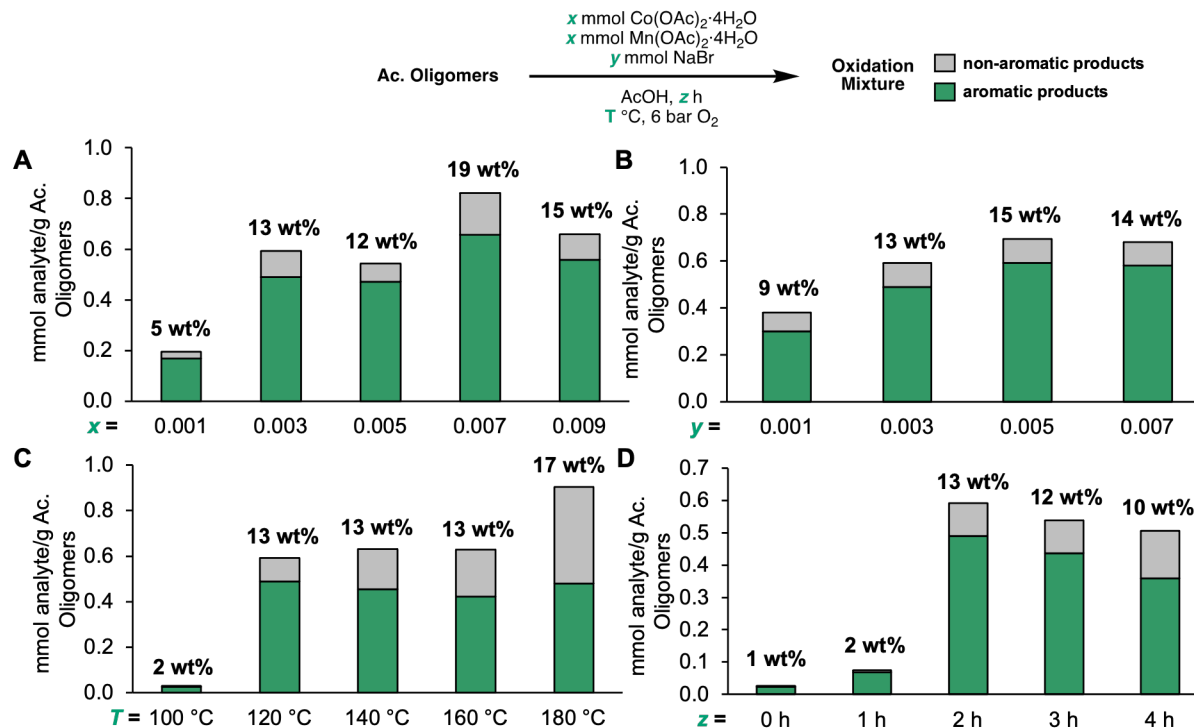


Catalytic strategy is effective for C–C bond cleavage in model compounds



Screening reaction conditions with C–C-linked dimers and oligomers

Gu, Palumbo, Bleem *et al.* *in review*



C–C-linked dimers and oligomers converted to aromatic monomers at mild conditions

- Yields are based on total lignin and higher yields will be accessible in flow

Proof-of-concept autoxidation catalysis on C–C-linked dimers and oligomers

Optimal conditions on poplar lignin oil with monomers removed

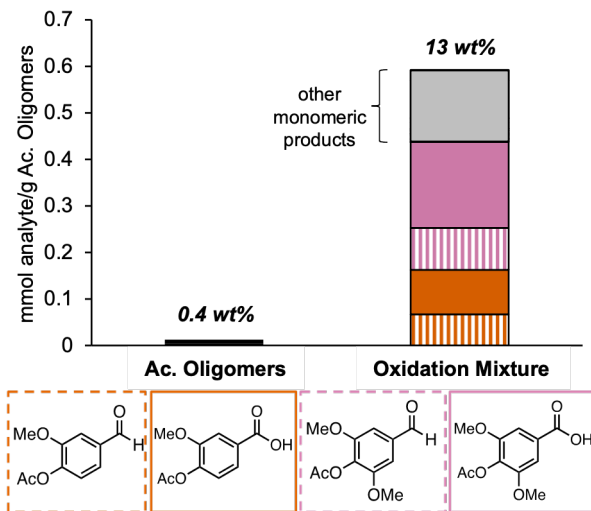
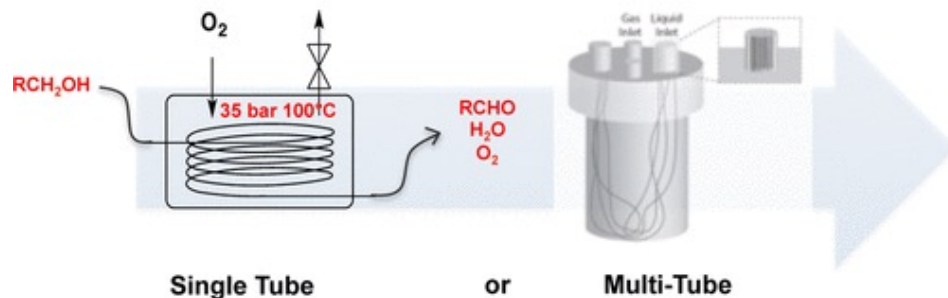


Image from Greene *et al.*, OPRD 2015



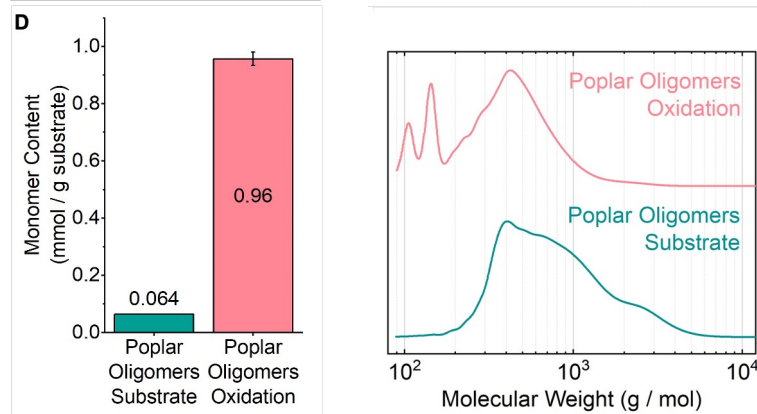
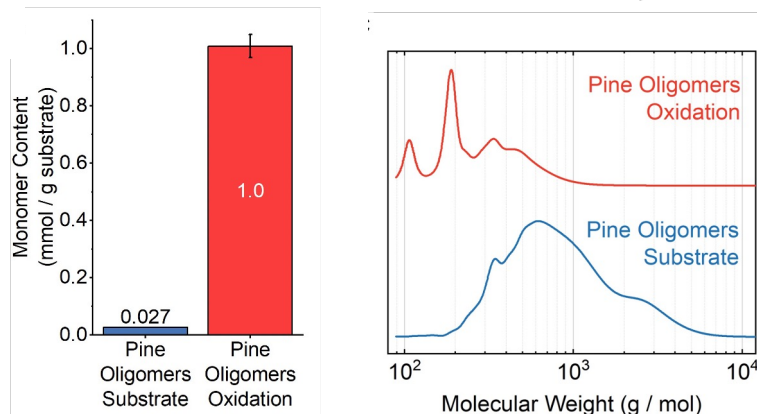
Collaborating with Shannon Stahl,
UW Madison on transition to flow

C–C-linked dimers and oligomers converted to aromatic monomers at mild conditions

- Flow chemistry is being developed now to improve selectivity to and yield of aromatic products
- Ongoing work to simplify stabilization reaction, including with acetic acid only
- Compounds are bio-available (see BLV presentation)

Methylation of phenols is another effective stabilization strategy

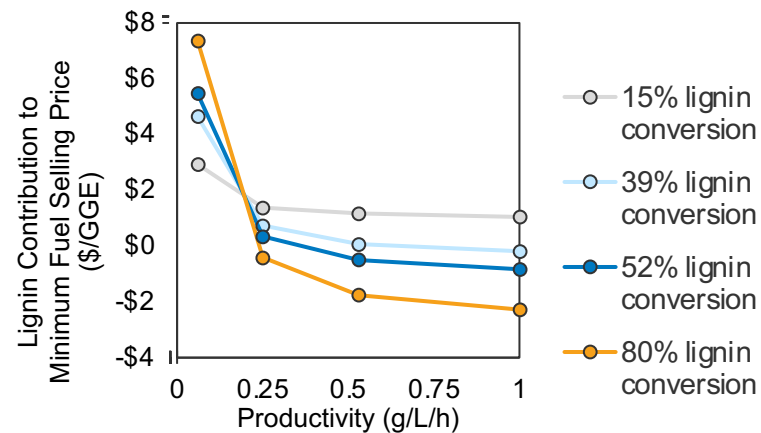
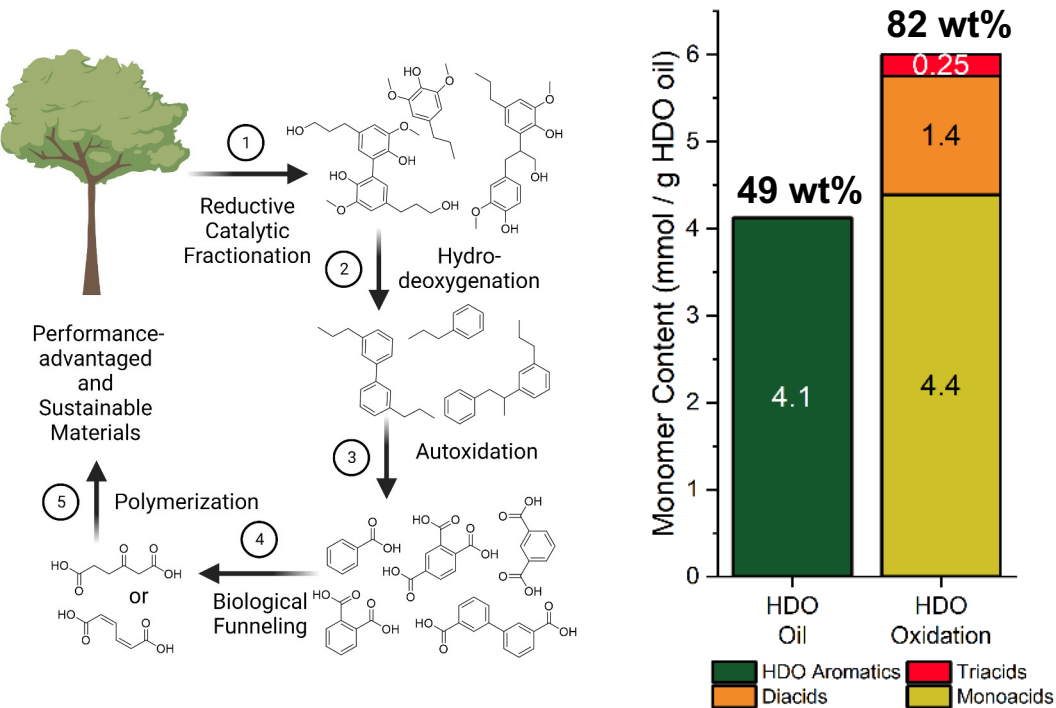
Methylated Oligomers $\xrightarrow{8 \text{ wt\% Mn(OAc)}_2 \cdot 4\text{H}_2\text{O}, 12 \text{ wt\% Zr(acac)}_4, \text{AcOH, O}_2 (6 \text{ bar}), 1.5 \text{ h}, 150 \text{ }^\circ\text{C}}$ Methylated Oxidation Monomers



Mn/Zr system shown to be effective with methylated lignin

- Ongoing work to determine if dimethyl carbonate can be used for methylation
- Demonstrated this chemistry to be effective with both pine and poplar RCF oil
- Methylated products are also bio-available (work with BLV)

Oligomers from lignin hydrodeoxygenation result in high product yields



These yields can enable a >\$2/gge contribution to biorefinery economics with biological funneling (BLV)

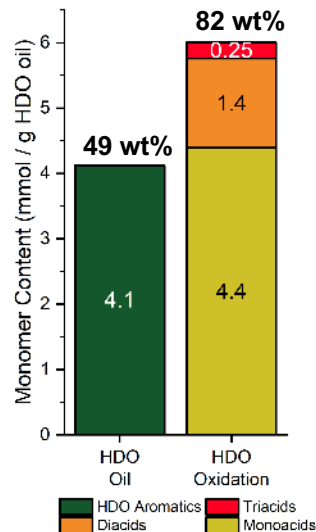
Impact

Scientific:

- First catalytic strategy for lignin depolymerization to aromatics to definitively cleave C–C bonds in lignin
- **Breaks the “monomer yield ceiling” set by C–O bond cleavage alone**
- **Enables “single valuable product” lignin valorization strategy**
- Publications and patent applications for lignin autoxidation will be online in 2023, with TEA and LCA ongoing
- Lignin analytical tools for high-throughput, automated lignin analysis

Industrial:

- Companies actively funding our lignin analytics capabilities and/or using our new methods in their labs
- Energy I-Corps team will be proposed around lignin autoxidation in early FY24
- Can enable **≥80% yield to a single product from lignin** and also be **complementary to LigSAF efforts** (light and heavy ends)



Honeywell
UOP



Borregaard

ExxonMobil



SUSTAINABLE FIBER
TECHNOLOGIES

Overall: Catalytic C–C bond cleavage can enable substantially higher-yielding lignin valorization processes

Summary

Overview

- Aim to develop viable processes to increase the theoretical monomer yield from lignin alongside robust analytics and accessible compound syntheses

Approach

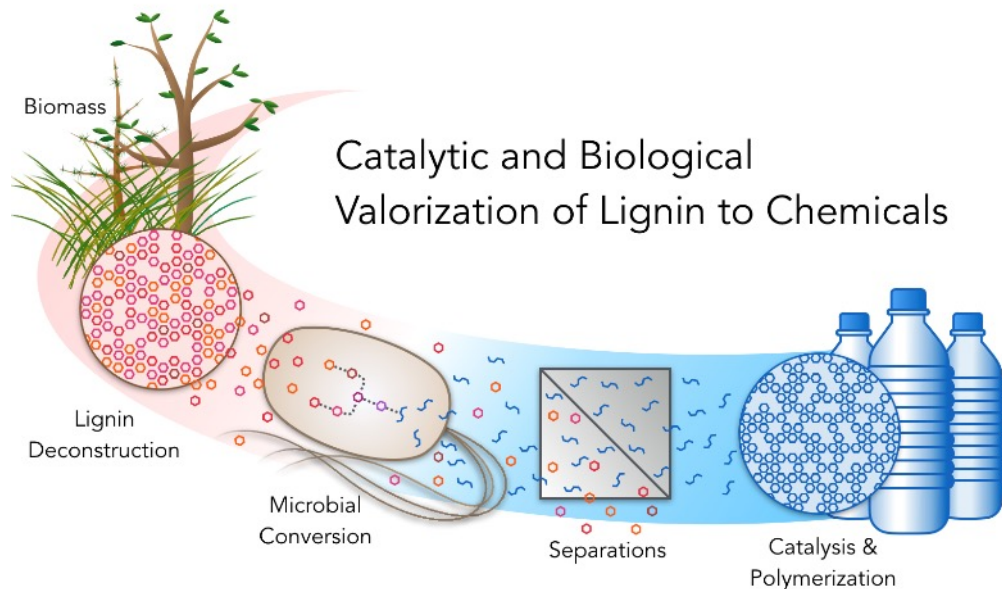
- Oxidation catalysis for C–C bond cleavage to make water-soluble, bio-available monomers

Progress and outcomes

- Developed an automated MS analysis pipeline for rapid lignin compound identification
- Demonstrated a robust method for C–C bond cleavage leveraging an industrial process from multiple lignin substrates

Collaborations and impact

- Work with all BETO-relevant lignin projects and with multiple industry partners



Beckham *et al.*, *Curr. Opin. Biotech.* 2016

Quad chart overview

	FY22 funding	Total Award
DOE Funding		

Project Partners

BETO projects: Lignin-First Biorefinery Development, Biological Lignin Valorization, Separations Consortium, Biochemical Platform Analysis

Nat’l labs: Oak Ridge National Laboratory

University collaborators: Massachusetts Institute of Technology, University of Wisconsin Madison

Project Goal

Develop industrially-relevant processes and tools for lignin valorization

End of Project Milestone

Deliver concentrated, lignin monomer-rich streams that contain 80% bio-available aromatic compounds to BLV, which can enable muconic or β -ketoadipic acid production at industrially relevant bioprocess parameters. Demonstrate a \$2/GGE and 70% reduction in GHG emissions relative to fossil-based adipic acid production.

Funding Mechanism

Bioenergy Technologies Office FY23 AOP Lab Call (DE-LC-000L015) – 2022

TRL at Project Start: 2
TRL at Project End: 4

Acknowledgements:

DOE Technology Managers Sonia Hammache and Beau Hoffman

NREL Contributors:

Brenna Black, David Brandner, Jeremy Bussard, Ryan Davis, Xueming Dong, Xu Du, Renee Happs, Stefan Haugen, Rui Katahira, Kelsey Kinley, Bruno Klein, Jacob Kruger, Megan Krysiak, Heather Mayes, Joel Miscall, Chad Palumbo, Michelle Reed, Lisa Stanley, Kevin Sullivan, Todd Vinzant

Collaborators:

Yuriy Román-Leshkov (MIT), John Ralph, Shannon Stahl (University of Wisconsin Madison)

Q&A

www.nrel.gov

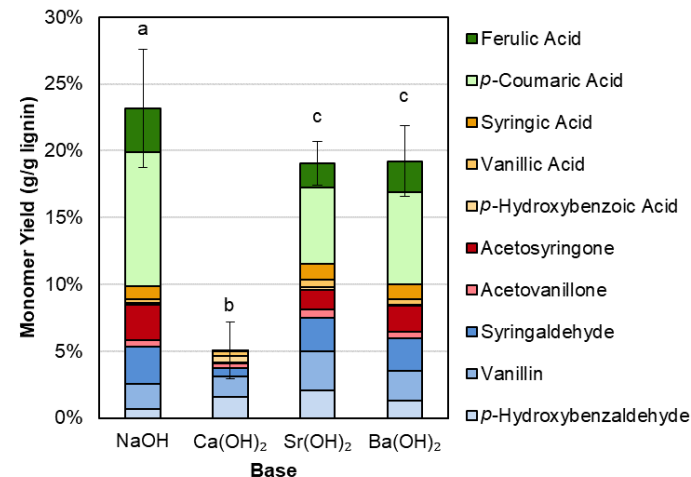
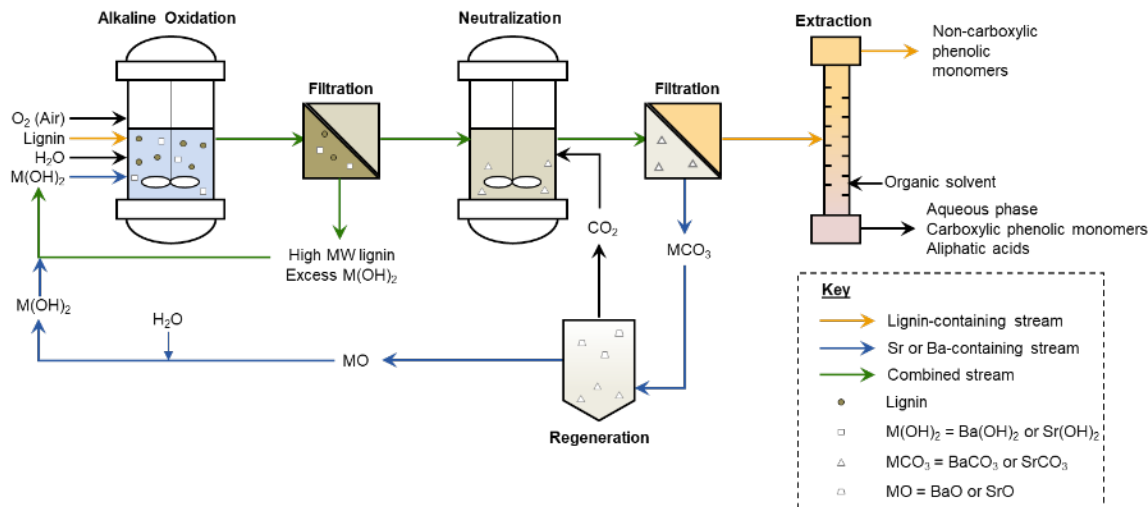
This work was authored in part by the National Renewable Energy Laboratory, operated by Alliance for Sustainable Energy, LLC, for the U.S. Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. Funding provided by U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office. The views expressed in the article do not necessarily represent the views of the DOE or the U.S. Government. The U.S. Government retains and the publisher, by accepting the article for publication, acknowledges that the U.S. Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this work, or allow others to do so, for U.S. Government purposes.



Additional Slides

Recoverable bases for base-catalyzed depolymerization of lignin

Kruger et al. *Green Chem.* 2022



NaOH use is a major contributor to environmental impacts of some lignin processes

- $Sr(OH)_2$ is a recoverable base that shows similar yields of monomeric aromatics to NaOH
- TEA and LCA both indicate promise in using recoverable bases to replace NaOH

Responses to previous reviewers' comments

- More details on the composition of the oxidized lignin mixtures will be helpful, but the literature suggests that complex mixtures of products will result.
- We agree that yield measurements and mass balances are critical. However, as the reviewers know, these measurements take a substantial amount of time to do rigorously for lignin. In terms of products, we fully agree that lignin oxidation products will be heterogeneous—this is why the Lignin Utilization project is working very closely with the Biological Lignin Valorization project to use microbes downstream of oxidative depolymerization to be able to produce high yield of single lignin-derived products.

Publications, patents, and presentations

Publications

In preparation or review

Chad T. Palumbo, Allison Z. Werner, Matthew S. Webber, David G. Brandner, Jeremy R. Bussard, Shannon S. Stahl, Yuriy Román-Leshkov, Gregg T. Beckham, High atom economy conversion of lignin into aromatic monomers through carbon–carbon bond cleavage, manuscript in preparation.

Chad T. Palumbo, Nina X. Gu, Alissa Bleem, Kevin P. Sullivan, Mikhail O. Konev, Rui Katahira, Lisa Stanley, Kelsey J. Ramirez, Stefan J. Haugen, Morgan A. Ingraham, Caroline R. Amendola, Shannon S. Stahl, Gregg T. Beckham, Catalytic carbon–carbon bond cleavage in lignin via manganese–zirconium-mediated autoxidation, manuscript in preparation.

Nina X. Gu, Chad T. Palumbo, Alissa C. Bleem, Kevin P. Sullivan, Stefan J. Haugen, Sean P. Woodworth, Kelsey J. Ramirez, Lisa Stanley, Rui Katahira, Shannon S. Stahl, Gregg T. Beckham, Autoxidation catalysis for carbon–carbon bond cleavage in lignin, in review.

Xu Du, Mikhail O. Konev, Kevin P. Sullivan, Rui Katahira, Chad T. Palumbo, Nina X. Gu, Sean P. Woodworth, David G. Brandner, Shannon S. Stahl, Gregg T. Beckham, Tandem acid-promoted and aerobic cleavage of C(OH)–C bonds with a polyoxometalate catalyst, in review.

Accepted

Xueming Dong, Heather B. Mayes, Kris Morreel, Rui Katahira, Yanding Li, John Ralph, Brenna A. Black, Gregg T. Beckham, Energy-resolved mass spectrometry as a tool for lignin identification, *ChemSusChem*, accepted.

Publications, patents, and presentations

2022

Jacob S. Kruger, Reagan J. Dreiling, Daniel G. Wilcox, Arik J. Ringsby, Katherine L. Noon, Camille K. Amador, David G. Brandner, Kelsey J. Ramirez, Stefan J. Haugen, Bruno C. Klein, Ryan Davis, Rebecca J. Hanes, Renee M. Happs, Nicholas S. Cleveland, Earl D. Christensen, Joel Miscall, Gregg T. Beckham, Lignin alkaline oxidation using reversibly-soluble bases, *Green Chem.* (2022), 24, 8733-8741.

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ROI-20-84	Method for Quantifying Inorganic Carbon in Solution
ROI-21-40	Process for converting CO ₂ into Conductive Carbons via Flash Joule Heating

Patents (provisional)

ROI-18-88	Heterogeneous Catalytic Oxidation of Lignin Carbon-Carbon Linkages
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